New Vaccines for the Prevention of Pneumococcal Infections

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Streptococcus pneumoniae is a major cause of acute otitis media, pneumonia, bacteremia, and meningitis. Because in recent years antibiotic-resistant pneumococcal strains have been emerging throughout the world, vaccination against pneumococcal infections has become more urgent. The capsular polysaccharide vaccine that has been available is neither immunogenic nor protective in young children and other immunocompromised patients. Several pneumococcal proteins have been proposed as candidate vaccines, but no human studies associated with them have been reported. Clinical trials of first-generation pneumococcal conjugate vaccines have shown that covalent coupling of pneumococcal capsular polysaccharides to protein carriers improves the immunogenicity of the polysaccharides. The protective efficacy of the conjugate vaccines against carriage, acute otitis media, and invasive infections is being studied.

Streptococcus pneumoniae (pneumococcus [Pnc]) is a common bacterial agent found in mild mucosal as well as severe systemic infections. Local infections, such as acute otitis media, are rather common; every child has at least one during the first 2 years of life (1), and Pnc is the causative agent in approximately half of the bacterial culture-positive cases (2). Pneumonia is another disease often caused by Pnc, both in industrialized and developing countries. Pneumonia, which causes more than one million deaths per year, is the most common cause of childhood death in the developing world (3); pneumococcal pneumonia is a serious problem among the elderly in industrialized countries. Pnc also causes frequent invasive infections, especially among children. In Finland, the incidence of bacteremic pneumococcal infections at 0 to 4 years of age has been 24.2 per 100,000 per year (4). The corresponding rate was 42 per 100,000 per year in Israel (5) and 66 per 100,000 per year in the United States (6). In addition to the young and the elderly, some of the other groups at increased risk for Pnc infection are patients with chronic cardiac or pulmonary diseases, immunocompromised patients, and especially persons with functional or anatomic asplenia (7).

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The treatment of recently emerged Pnc strains that are resistant to penicillin and other antibiotics (8) is becoming a challenge. Because of the high rates of illness and death associated with pneumococcal infections and the rapidly increasing resistance of organisms that cause these infections to antimicrobial drugs, development and use of effective pneumococcal vaccines is of high priority. The progress has been rapid; in addition to polysaccharide(PS)-protein conjugate vaccines, vaccines containing pneumococcal proteins are also being developed.

Pneumococcal Capsular Polysaccharide Vaccine

Pnc can be divided into at least 90 serotypes according to the structure of the PS in the capsule surrounding the bacterium. The capsule seems to be the most important virulence factor; all strains isolated from infections are encapsulated. The capsule helps the bacterium escape the host defense mechanisms. However, only a small fraction of all capsular types are common causes of pneumococcal infections. The list of the most common groups/types (4, 6, 7, 9, 14, 18, 19, and 23) that cause childhood infections is similar in most parts of the world. Types 1 and 5 are, however, more common in the developing world than in industrialized countries (9).

Antibodies to capsular PSs protect from infection by opsonizing Pnc for phagocytosis by neutrophils. A capsular PS vaccine containing 23 of the most common serotypes/ groups has proven protective in immunocompetent adults and in some groups at risk (7,10,11); it has also been shown to have an impact on death rates due to pneumonia in Papua New Guinea (12). Among the immunocompromised and in preventing acute otitis media, (13) its efficacy has been only marginal.

The reason for the vaccine's poor immunogenicity and its lack of efficacy in children is thought to be the nature of the PS antigen. PS antigens are type 2 T-cell independent (TI) antigens, which stimulate mature B cells without the help of T cells. In humans, the B cells of newborns do not respond to most of the PS antigens. Responsiveness develops only slowly during the first years of life. Furthermore, the TI antigens do not induce immunologic memory and the maturation of the immune response; anti-PS antibodies have low avidity and the switch from one isotype to another does not happen even after repeated immunizations. The TI antigens induce mainly IgM responses, especially in mice. However, in humans the response also contains the IgG and IgA components (14). Furthermore, the IgG response to PS antigens contains a greater proportion of IgG2 (15,16) than found in a response to protein antigens. The lack of memory has some important implications for the vaccination. Because of the rapid decline of antibodies, revaccination is often necessary (7).

Pneumococcal Protein Vaccine Candidates

Several ways have been and are being tried to solve the problem of poor immunogenicity of pneumococcal PS vaccines in infancy. In addition to the capsule, other pneumococcal virulence factors have been considered as promising vaccine candidates or as carrier proteins in pneumococcal conjugate vaccines (see above). The prime vaccine candidates are enzymes and toxins that are excreted or released after the bacterium has autolyzed or surface proteins whose exact functions are not known. Pneumococcal proteins studied as potential vaccines include neuramididase, autolysin, pneumolysin, pneumococcal surface protein A (PspA), and pneumococcal surface adhesin A (PsaA) (17-19).

Pneumolysin is a cytolytic toxin produced by all types of Pnc. In mice, immunization with inactivated pneumolysin or recombinant pneumolysin toxoid offers at least partial protection or enhanced survival when challenged with Pnc (20,21). PspA is a surface protein present in all clinically relevant pneumococcal strains. PspAs from different pneumococcal strains vary serologically. However, many PspA antibodies cross-react with PspAs from unrelated strains. Furthermore, active immunization of mice with PspA generates protective immune response against diverse pneumococcal strains (22). Truncated PspAs, expressed as recombinant proteins, are also immunogenic in mice and can elicit cross-protection (18).

Pneumococcal Conjugate Vaccines

Another approach to solving the poor immunogenicity of the capsular PS antigens has already moved to the clinical phase-III trials. This approach is based on the 1929 findings of Goebel and Avery (23), who showed that covalent coupling of haptens to a protein carrier improves the immunogenicity of the hapten. In this way, the anti-PS response gets T-cell dependent characters: there is development of immunologic memory and maturation of the immune response. This is seen as an increase in the antibody concentrations and the antibody affinity and as a switch in the isotype distribution after repeated immunizations. This approach has been used successfully to prepare vaccines against *Haemophilus influenzae* type b (Hib); the incidence of Hib infection has decreased drastically wherever these conjugate vaccines have been used (24).

The PS antigen in a conjugate vaccine seems to benefit at least partly from the immunologic characters of the carrier protein. The protein is presented as peptides in association with the major histocompatibility complex class II molecules on the surface of the antigen-presenting cells. This stimulates the T-helper cells, which then stimulate adjacent B cells for antibody production and maturation into memory cells. Development of immunologic memory means that the protection does not depend solely on the existing antibody concentration. Instead, the vaccinated persons can respond with a rapid, high, and effective antibody response to colonization or invasion by the respective Pnc type. Studies in Finland suggest that this indeed happens: the efficacy of an Hib conjugate vaccine, PRP-D, was more than 90% in early infancy, even though a large proportion of the infants did not have measurable antibody response after the primary course of immunization (25). A study in the United Kingdom suggests that the carriage of Hib indeed induces a high "booster type" immune response (26).

Conjugation of the PS to a protein carrier has repeatedly been shown to work with Hib; vaccines based on the same principle would also decrease the number of different infections caused by Pnc. Four vaccine manufacturers have prepared pneumococcal conjugate vaccines with basically the same approaches as the Hib conjugates (Table 1). PncOMPC vaccine contains PSs from seven serotypes conjugated to the meningococcal outer membrane protein complex (27). The PncCRM vaccine contains either oligosaccharides (OS) or PSs coupled to a nontoxic mutant diphtheria toxin CRM197. The PS-containing conjugate vaccine is at present heptavalent (28), but it is possible to add types 1 and 5 to the product intended for use in developing countries. The PncT vaccine contains eight PSs coupled to tetanus toxoid, and the PncD product contains the same PSs coupled to diphtheria toxoid (29). Besides these formulations, several other approaches have been tested in animals. These include conjugates using pneumolysoid (30), pertussis toxin (31), and salmonella protein (32) as a carrier. Recently, small peptides selected on the basis of T-cell stimulating properties have also been coupled to pneumococcal PS to form conjugate vaccines (33).

Table 1. Pneumococcal conjugate vaccines in phase-II and phase-III trials

Vaccine	Serotype	Carrier	Manufacturer
PncCRM	4, 6B, 9V, 14, 18C, 19F, 23F	CRM197	Wyeth-Lederle Vaccines and Pediatrics
PncD	3, 4, 6B, 9V, 14, 18C, 19F, 23	Diphtheria toxoid	Connaught Laboratories
PncT	3, 4, 6B, 9V, 14, 18C, 19F, 23	Tetanus toxoid	Pasteur Merieux Serums & Vaccins
PncOMPC	4, 6B, 9V, 14, 18C, 19F, 23F	Meningo-coccal OMPC	Merck Research Laboratories

CRM = CRM197, a nontoxic variant of diphtheria toxin; D = diphtheria toxoid; T = tetanus toxoid; OMPC = outer membrane protein complex

Preclinical Testing

Before human trials, these conjugates were immunogenic and protective in animals, including mice, infant monkeys, and chinchillas (34-37). All these studies indicate that conjugate vaccines have greater immunogenicity than pneumococcal PS vaccines. Even though animal studies can tell if the conjugate vaccine is immunogenic and evokes a T-cell dependent response, the final proof of conjugate vaccines' superior immunogenicity and efficacy over PS vaccines comes only from human studies. So far no animal model can mimic human immunogenicity and efficacy studies.

Clinical Testing

Pneumococcal conjugates of all the manufacturers mentioned in Table 1 have now been tested in phase-I and phase-II studies. The first human studies were done in adults with mono- or bivalent conjugates and showed that the conjugates were at least as immunogenic as the PS vaccine. Since then, up to eight valent vaccines have been used in human studies, also among infants.

Adults and Toddlers

To show that they are safe and immunogenic, pneumococcal conjugates were first given to small numbers of adults and toddlers. Most of the reported studies have been conducted with mono- to tetravalent vaccines. The PncOMPC studies in adults show that the conjugate vaccine was well tolerated but not more immunogenic than the PS vaccine (38,39). One possible reason might be the low dose (1µg to 5µg of each conjugate) used in these studies. Different formulations of PncCRM containing either PS or OS linked to CRM197 have been tested in adults. All were well tolerated and evoked a comparable immune response (40). This was confirmed in a study in which heptavalent OS conjugate was immunogenic (28). Results of immunizing adults with PncT or PncD conjugates have been reported in two studies; both vaccines were more immunogenic than the PS vaccine (41,42). The Finnish study with tetravalent PncT and PncD showed that these conjugates can also evoke a mucosal antibody response (42).

PncOMPC vaccine was given to 31 Finnish children at 24 months, and 10 of them also received it at 26 months. The primary response was only slightly higher than to the PS vaccine, but after the second dose a booster type response was seen in most of the vaccinees (43). Studies conducted during the second year of life showed that the heptavalent PncOMPC conjugate was more immunogenic than the PS vaccine (44,45). Different formulations of PncCRM have also been tested in toddlers (46). Conjugates were more immunogenic than the PS vaccine; furthermore, the PS conjugate was more immunogenic than the OS conjugate. One study showed a good booster response to PS vaccine after primary immunization with pentavalent PS-based PncCRM (47). The PncT and PncD conjugates have also proven immunogenic in toddlers. A Finnish study compared 3-µg and 10-µg doses at 24 months, and a U.S. study used 10-µg doses of type 19F conjugates with PS vaccine booster doses (48).

Infants

Keyserling et al. (49) have compared different dosages of type 14 PS containing monovalent PncOMPC vaccine in infants and shown that 2.5µg to 5µg of type 14 PS in the conjugate gave better responses than the lower doses. A Finnish study (50) showed that a primary series of three doses of tetravalent PncOMPC at 2, 4, and 6 months was better than two doses at 4 and 6 months. Furthermore, a booster dose of PncOMPC given at 14 months evoked a secondary response to all PS types. Concomitant administration of PncOMPC with routine infant immunizations does not seem to have an effect on either anti-Hib or anti-Pnc PS antibody responses (51). The heptavalent PncOMPC formulation is as immunogenic as the previous formulations with fewer serotypes (27).

Åhman et al. have shown that the pentavalent PncCRM vaccine containing OS derived from pneumococcal capsule was immunogenic and tolerable in infants (52). The same children developed a good antibody response when boostered with PS vaccine at 24 months, suggesting that the immunologic priming had been good even if the antibody response to the primary series had remained rather low (53). The PS-based PncCRM has been shown to be more immunogenic than OS conjugates also in infancy (54). A Gambian study evaluated the pentavalent PncCRM conjugate (PS-based) in a developing country when given at 2, 3, and 4 or at 2 and 4 months. The vaccine was immunogenic and well tolerated; the schedule of three doses was better than the two-dose schedule (55).

A Finnish study compared three dosages of 1µg to 10µg of each PS in tetravalent PncT and PncD conjugates when administered at 2, 4, and 6 months. These vaccines were immunogenic in infancy, and no difference could be shown between PncT and PncD. The response after a primary series to PncD, but not to PncT, was dose dependent (56). The children immunized with PncD in infancy had a booster response after reimmunization with either PncD or pneumococcal PS vaccine at 14 months (57). All who received PncT were boostered with PS vaccine, and the response was dose dependent; children that had received 10-µg doses of PncT during the primary immunization had the lowest mean booster responses (58). Another Finnish study showed that octavalent (types 3, 4, 6B, 9V, 14, 18C, 19F, and 23F) PncD (3µg of each PS) and PncT (1µg of each PS) induced immune responses similar to the respective tetravalent formulations (29). An Icelandic study showed that the octavalent vaccine was immunogenic in infants when given at 3, 4, and 6 months and that the IgG anti-PS concentrations correlated with the opsonic activity (59).

Because no study has directly compared different pneumococcal conjugate vaccines,

Table 2. Antibody response of Finnish infants to pneumococcal conjugate vaccines administered at 2, 4, and 6 months of age*.

	Geometric Mean of the Anti-PNC PS (µg/ml)								
	Type 6B		Type 14		Type 19F		Type 23F		
Vaccine	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Ref.
PncOMPC	0.17	1.30	0.42	8.27	0.34	9.85	0.28	1.90	(50)
PncCRM	0.25	0.50	0.30	2.49	0.46	1.13	0.18	0.83	(52)
PncT01-4	0.25	0.89	0.24	2.84	0.36	3.73	0.18	0.82	(56)
PncT01-8	0.20	1.28	0.30	2.56	0.56	4.23	0.22	1.03	(29)
PncD03-4	0.26	0.88	0.44	2.20	0.43	5.29	0.21	0.67	(56)
PncD03-8	0.17	1.44	0.31	4.62	0.37	4.94	0.24	1.07	(29)

PncOMP = tetravalent conjugate vaccine with a meningococcal outer membrane protein complex as a carrier
PncCRM = pentavalent oligosaccharide conjugate vaccine with CRM197 protein as a carrier
PncT01-4 = tetravalent conjugate vaccine with tetanus toxoid carrier; 1 µg of each of four polysaccharides
PncT01-8 = tetravalent conjugate vaccine with tetanus toxoid carrier; 1 µg of each of four polysaccharides
PncD03-4 = tetravalent conjugate vaccine with diphtheria toxoid carrier; 3 µg of each of four polysaccharides
PncD03-8 = octavalent conjugate vaccine with diphtheria toxoid carrier; 3 µg of each of four polysaccharides
*Serum samples are taken before immunization (pre) and at 7 months (post). The data have been gathered from separate studies done in the same population.

the comparison has to rely on data from separate studies. A Finnish group has analyzed the antibody response in adults, toddlers, and infants to all four types of Pnc conjugates. This comparison shows that there are vaccine- and type-specific differences in the antibody responses (Table 2). However, none of the vaccines used in these studies have the composition suggested in the phase-III trials (29,50,52,56).

Comparing the data from different studies is difficult because there can be interlaboratory variation in the enzyme-linked immunosorbent assay results. The Centers for Disease Control and Prevention, Food and Drug Administration, and World Health Organization are working on a standardized anti-Pnc PS assay, which will, if not eliminate, at least reduce the impact of this problem. A standard serum (60) to be used in all laboratories is distributed by Center for Biologics Evaluation and Research/Food and Drug Administration.

Pneumococcal Conjugates and the Carriage of Pnc

Experience with the Hib conjugates (24) suggests that Pnc conjugate vaccines could also reduce the number of carriers of the vaccine types and in this way decrease the spread of bacteria. The results from the only reported study are encouraging. The PncOMPC vaccine decreased the carriage rate among toddlers, while the pneumococcal PS vaccine did not (45). Importantly, the carriage of antibiotic-resistant Pnc also decreased (61).

Efficacy Studies

Phase-III studies with the heptavalent formulations of PncOMPC and PncCRM are ongoing or being started. These studies look at prevention of carriage, acute otitis media, or invasive Pnc infection caused by Pnc of the vaccine serotypes. Furthermore, there are several plans for studying the effect of Pnc conjugates on Pnc invasive infection and pneumonia in developing countries.

Questions to Be Answered in the Future

We do not know if conjugate vaccines can really prevent Pnc infections better than the PS vaccine. We hope that the new vaccines can prevent several types of infections, from symptomless Pnc carriage to serious invasive infections with high death rates. It is quite probable that the protective immune response needed is different for each type of infection. We do not know if parenterally administered vaccine can prevent carriage or mucosal infections such as acute otitis media. It is still unknown whether a mucosal immune response is needed or whether transudation of antibodies from the serum is enough for protection against local infection. Saliva samples of infants immunized with Hib conjugate vaccines contain secretory IgA but also IgG, which has most probably transudated from serum (62). In an infant rat Hib colonization model, both secretory IgA and serum derived IgG decreased colonization (63). Furthermore, animal experiments suggest that immune response evoked by parenteral administration of a conjugate vaccine would alone protect against acute otitis media (64). In addition, passive immunization of infants with hyperimmune serum pool containing antibodies to pneumococcal PS-induced protection against pneumococcal acute otitis media suggests that protection is offered when high enough serum antibody concentrations are gained (65). At present, there are no data to show which antibody concentrations are needed for protection. Deciding about the protective concentration might be difficult because the development of immunologic memory is an important factor; the protection does not solely depend on the existing antibody concentration.

Most phase-II studies have used a schedule of two or three doses of Pnc conjugate vaccine in infancy (usually at 2, 4, and 6 months) and a booster dose of either conjugate or Pnc PS at the second year of life. The need for a booster dose at the second year is not known; this information would be important especially for planning the vaccination schedules for developing countries, where administering a booster dose can be problematic. The experience from the Hib conjugates suggests that a booster dose might not be needed; the United Kingdom has successfully used a schedule of three doses at 3, 4, and 5 months without a booster dose (66).

Reduction of pneumococcal infections among the elderly would probably increase the quality of their lives. The immunogenicity of pneumococcal PS vaccine in this age group has been satisfactory (67,68). An Hib conjugate (PRP-D) has proven more immunogenic than the Hib PS vaccine in the elderly (69). However, the immune response to PncCRM was not better than to the Pnc PS vaccine, and no booster response was seen (70). Studies with other pneumococcal conjugates in the elderly have not been reported.

Because pneumococcal infections of very young infants are a problem in developing countries, several groups have studied the possibility of maternal immunization with pneumococcal vaccines (71,72). So far only PS vaccines have been used; even though these vaccines are immunogenic in pregnant mothers, the immunity transferred to the neonate is not very long lasting. If the conjugate vaccines induce higher antibody concentrations in mothers, the concentration of passively acquired antibody in the baby would stay high for a longer time. The Hib conjugate vaccines induce good responses in mothers and, consequently, long-lasting protective concentrations in infants born to these mothers (73,74). The effect of simultaneous maternal tetanus immunization, especially if conjugates with a tetanus toxoid carrier are used, and the effect of high maternal antibody level on the antibody responses of the infants have to be determined.

By the year 2000, we may have pneumococcal conjugate vaccines to include in routine childhood immunization programs. The price of the conjugate vaccines has been so high that their use throughout the world has not been possible. An important challenge in developing of pneumococcal conjugate vaccines is to reduce the costs of manufacturing so that all children can benefit from them.

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